AD-A264 848

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REPORT DOCUMENTATION PAGE

Form Approved OMB No 0704-0188

1a REPORT SECURITY CLASSIFICATION (U)	1b RESTRICTIVE MARKINGS NA
2a SECURITY CLASSIFICATION AUTHORITY NA	3 DISTRIBUTION/AVAILABILITY OF REPORT Distribution unlimited
2b DECLASSIFICATION/DOWNGRADING SCHEDULE NA	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) Princeton University	5 MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a NAME OF PERFORMING ORGANIZATION Princeton University; 6b OFFICE SYMBOL (If applicable) NA	7a NAME OF MONITORING ORGANIZATION Office of Naval Research
6c ADDRESS (City, State, and ZIP Code) Princeton, NJ 08544	7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street, Arlington, VA 22217-5000
8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research; 8b OFFICE SYMBOL (If applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N-00014-90-J-1702
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street, Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO 61153N; PROJECT NO RR04108; TASK NO 441k818; WORK UNIT ACCESSION NO

11 TITLE (Include Security Classification)

(U) Lipid Dependent Mechanisms of Protein Pump Activity

12 PERSONAL AUTHOR(S)

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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT
Final	FROM 6/90 TO 11/92	29/04/93

93-11522

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
08			Lipid effect; protein pumps; membrane protein

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The overall objectives of the grant were to investigate the relationship between the activity of membrane proteins, such as protein pumps and channels, and the elastic curvature properties of the imbedding lipid bilayer. The goal was to understand if lipid composition modulates the protein activity via a coupling to the lipid monolayer elastic energies. This involved investigation both of the physical properties of lipid systems and measurement of the effects upon specific proteins. Progress on the following specific objectives are summarized:

1) To develop techniques for measuring lipid curvature elastic properties.
2) To investigate if the bilayer spontaneous curvature is regulated in bacterial cells.
3) To correlate ion-pump and channel activity with the spontaneous curvature.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION (U)
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. Igor Vodyanoy	22b TELEPHONE (Include Area Code) 202-696-4056; 22c OFFICE SYMBOL ONR

DD Form 1473, JUN 86 Previous editions are obsolete SECURITY CLASSIFICATION OF THIS PAGE (U)

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FINAL TECHNICAL REPORT

GRANT #: N00014-90-J-1702

PRINCIPAL INVESTIGATOR: Sol M. Gruner

INSTITUTION: Princeton University

GRANT TITLE: Lipid Dependent Mechanisms of Protein Pump Activity

GRANT PERIOD: 06/01/90 – 11/30/92

I) Summary

The objectives of the grant were to investigate the relationship between the activity of membrane proteins, such as pumps and channels, and the elastic curvature properties of the imbedding lipid bilayer. The goal was to understand if the lipid composition affects protein activity via a coupling to the bilayer elastic constants. The specific objectives were:

1) To develop experimental methods of measuring membrane elastic properties and the interaction with proteins.
2) To correlate protein pump activity and channel activity with membrane elastic properties.
3) To investigate the relationship between lipid composition and the spontaneous curvature of native membranes.

The first two specific objectives were successfully met, while the third objective is delayed pending receipt of data from collaborators. These results are summarized below.

II) Experimental Methods

The goal here was to use x-ray and neutron diffraction methods to investigate the validity of the physical theory of membrane elastic curvature, as described in detail in Gruner, 1989; 1991; 1992a; 1992b. The primary papers describing the x-ray and neutron diffraction investigations are Turner and Gruner 1992; Turner et al, 1992a; Turner et al, 1992b. The first of these three papers describes the development and application of Fourier methods to reconstruct the structure of the inverse hexagonal phase of certain membrane lipids. The reconstructions are used to show that the density variations in the lipid hydrocarbon diverge as the lamellar-nonlamellar phase transition is approached from high temperature, in agreement with the idea that the lamellar phase represents a state of frustrated curvature. This is important because this curvature is the basis of the coupling of curvature-prone lipid bilayers to proteins imbedded in those bilayers (e.g., see Gruner, 1991).

Turner et al, 1992a describes the combination of x-ray and neutron diffraction to demonstrate that alkanes in the inverted hexagonal phase lattice preferentially occupies hydrocarbon-stressed volumes amongst the lipid chains. This result is significant because it proves that the large effect of short alkanes upon the lamellar-nonlamellar phase transition is due to relaxation of the curvature frustration in curvature-prone bilayer phases, again lending support to the notion that these curvature-frustrated forces are present to exert torques upon imbedded membrane proteins.

The third paper (Turner et al, 1992b) reports on an x-ray diffraction study which is used to extract the elastic constants of lipid layers. The significance of this paper is that it provides numerical estimates of these elastic constants. These, in turn, set limits on the energy available for conformational action on proteins in curvature-frustrated bilayers.

III) Protein Channels and Lipid Curvature

The second specific goal was to use a model protein system to directly demonstrate that lipid spontaneous curvature couples to protein channel function. This was accomplished via the use of alamethicin in a black-lipid membrane (BLM) geometry. These results are described in Keller et al, 1993, which is now in press. The experiment consisted of incorporating alamethicin into BLMs of different lipid compositions and then investigating the electrical channel properties which resulted. The x-ray methods developed earlier were used to characterize the spontaneous curvature of the various lipid compositions. Alamethicin exhibits a series of discrete, well-defined conductance states at fixed transbilayer potential. It was shown that the relative probability of population of the different conductance states varied systematically with the spontaneous curvature. Moreover, when similar, but different lipids were used to make the BLM, compositions with identical curvatures yielded identical effects on the conductance states. These results are highly significant and demonstrate that the physical forces present in curvature-frustrated bilayers coupled to activity in the alamethicin system. Of course, if these forces affect one protein channel, then it is reasonable to assume that other protein channels will also be affected.

IV) Regulation of Native Membrane Lipid Compositions

This goal was to investigate if bacteria regulate the spontaneous curvature of their membranes via control of the lipid composition. This would be reasonable if lipid spontaneous curvature is an important variable affecting membrane proteins vital to the organism. Completion of this goal has been delayed by difficulties in obtaining lipid extracts and some NMR results from our foreign collaborators. Even so, the majority of the work associated with this last objective is completed, as summarized in the letter to Dr. Igor Vodanoy of ONR (dated 9 March 1992). Basically, the results show that the spontaneous curvatures of lipids extracted from mycoplasma membranes cluster tightly, even under growth conditions which result in membrane compositions which are quite varied. Unequivocal interpretation of the data requires NMR verification of the phase assignments, and calibration of the lipid curvatures against some pure lipid fractions extracted from the lipid mixtures. These steps are being performed, albeit slowly, by the Swedish collaborators who grew the bacteria. We are hopeful of soon obtaining the last of the lipid extracts required to to complete interpretation of the data. Even though this information will be obtained beyond the expiration data of the ONR grant, we intend to complete the work, since what remains to be done is primarily analysis and synthesis of the data.

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